

# Genetic diversity and genetic differentiation of natural *Pinus koraiensis* population

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**Abstract:** Genetic diversities and genetic differentiations of the four *Pinus koraiensis* populations (Gaofeng Forestry Farm at Tangwanghe in Yichun City, Erdaobaihe Town in the Changbai Mountains, Shengshan Forestry Farm in Heihe City, in China, and the suburb of Vladivostok City in Russia) were analyzed by using ISSR-PCR technique. The results of 15 primers amplification showed that the ratio of polymorphic site of *P. koraiensis* population was 60.7%. Each primer had 3.6 polymorphic sites. The diversity levels of the four *P. koraiensis* populations were rather higher compared with those of other *Pinaceae* species. The genetic diversity levels of *P. koraiensis* populations grown in the center region were higher than those grown in the edge zone. The genetic diversity of *P. koraiensis* mainly came from the interior of the population, accounting for 73% of total genetic diversity. There were no positive correlation between genetic distances and geographical distances for the four *P. koraiensis* populations. The gradual decrease of natural distribution region of *P. koraiensis* was due to anthropic destroy and environmental factors (i.e. fire and wind throw), rather than the lower genetic diversity.

**Keywords:** Genetic differentiation; Genetic diversity; ISSR; *Pinus koraiensis*

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## Introduction

ISSR (inter simple sequence repeat) technique belongs to dominant marker with RAPD. Amplification products of ISSR with fewer segment had higher specialty and stability compared with that of RAPD. As a method of molecule marker, ISSR was adopted initially in the construction of genetic linkage map of crop and assisting breeding of ripe, wheat, sugar beet, and so on (Jiang *et al.* 1998, Arcade *et al.* 2000). However, few studies were conducted on genetic diversity of forest by this method. Tsumura *et al.* (1996) reported that the method could be introduced in study of construction of genetic linkage map and genetic diversity of population for coniferous trees. Amplification products of ISSR had stronger specialty and higher steady although they had fewer polymorphic loci (Qianwei *et al.* 2000). Thus ISSR method had been gradually used in study of plant genetic diversity (Jonsson *et al.* 1996)

Korean pine (*Pinus koraiensis*) is an important economic tree species in northeast of China for its excellent timber quality and delicious seeds. As the constructive community of broad-leaved/Korean pine forest, the typical climax vegetation in northeast of China, Korean pine has higher ecological value. It is difficult to restore Korean pine forests due to its slow growth and reproduction, late fruit and uneasy natural regeneration. In recent years, natural forest of Korean pine has been destroyed seriously and its natural growth has declined significantly. In this study, the genetic diversity level and model of genetic differentiation in

natural population of Korean pine were analyzed by ISSR. Geographic variation and evolutionary mechanism of Korean pine population were further discussed. The research result could provide a theoretic basis for better protecting and utilizing forest resources.

## Materials and methods

### Materials

Totally 87 samples were selected separately from three natural Korean pine populations of age in rang of 80–120 in northeast of China: (A) Gaofeng Forestry Farm at Tangwanghe in Yichun City, Heilongjiang Province, (B) Erdaobaihe Town in the Changbai Mountains, Jilin Province, (C) Shengshan Forestry Farm in Heihe City, Heilongjiang Province, and a natural Korean pine population (D) in suburb of Vladivostok City, Russia (Fig. 1). The average distance between sampled trees was at least 50 m. The fresh young needles were stored at –70°C.



**Fig.1 Sampled sites of Korean pine**  
A. Shengshan; B. Tangwanghe; C. Erdaobaihe; D. Vladivostok

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## Methods

### Extraction and purification of DNA

Sample DNA was extracted and purified by traditional CTAB method.

### Primer screening

Fifteen primers with better amplification effect were screened from 96 ISSR primers provided by Colombia University in Canada (Table 1) (Feng *et al.* 2004).

**Table 1. Sequences of 15 primers**

Primers	Sequences	Number of polymorphic site	Total sites
834	(AG) <sub>8</sub> YT	2	5
853	(TC) <sub>8</sub> RT	2	4
818	(TG) <sub>8</sub> C	7	7
808	(TG) <sub>8</sub> T	2	5
848	(CA) <sub>8</sub> BG	3	6
828	(TG) <sub>8</sub> A	4	6
823	(TC) <sub>8</sub> C	3	4
836	(AG) <sub>8</sub> Y	4	6
822	(TC) <sub>8</sub> A	1	3
812	(GA) <sub>8</sub> A	8	12
811	(CA) <sub>8</sub> C	4	7
807	(AG) <sub>8</sub> T	2	6
827	(AC) <sub>8</sub> G	10	12
873	(GACA) <sub>8</sub>	1	3
820	(GT) <sub>8</sub> C	3	8
Total		54	89

### PCR amplification

PCR amplification was performed at 94°C for 5 min, 94°C for 30 s, 52°C for 45 s, then 72°C for 2 min, 40 circles, and finally expanded at 72°C for 7 min (Tsumura *et al.* 1996). TaqDNA polymerase was provided by PROMEGA Company, and primers and dNTP were provided by TaKaRa. Every primer had a blank control with sterile deionized water to eliminate systematic error.

The amplified result was analyzed by man-made reading and calculating ratio of polymorphic site (*P*) according to the following equation.

$$P = (\text{total reading} - \text{mutual reading}) / \text{total reading}$$

The genetic diversity index of every population and degree of genetic differentiation in inter-population were analyzed by PopGen Software.

## Results

Fifteen primers amplified at least one polymorphic site were screened in this study. All the samples (87) from four sampled sites were steadily amplified separately. There were significant differences in the number of amplification segment, ranging between 3 and 12, with a size of between 400 bp and 2 000 bp. Fifteen primers were amplified 89 sites, including 54 polymorphic sites and 35 consensus sites. The ratio of polymorphic sites (*P*) was 60.70%, with an average of 3.6 polymorphic sites each primer.

### Ratio of polymorphic site

The *P* values of four populations were also lower than total *P* value. *P* value of Korean pine population at Tangwanghe was the highest, followed by that at Erdaobaihe and Heihe, and lowest at Vladivostok (Table 2).

**Table 2. The ratio of polymorphic site amplified by 15 primers for four Korean pine populations**

Populations	Number of samples	Number of polymorphic site	Number of total site	Ratio of polymorphic site
Vladivostok	18	35	76	46.05%
Tangwanghe	26	49	81	60.49%
Heihe	20	38	77	49.35%
Erdaobaihe	23	44	82	53.66%
Total	87	54	89	60.70%

### Genetic diversities of different Korean pine populations

Genetic diversity levels of four populations of Korean pine were analyzed by Nei and Shannon phenotype diversity indexes. Shannon indexes were higher than Nei indexes in the four populations (Table 3). The order of genetic diversity levels from big to small was Tangwanghe, Erdaobaihe, Heihe and Vladivostok whatever analyzing by Nei or Shannon indexes. The levels of genetic diversities of Korean pine grown in the centre zone were higher than those in the edge zone.

**Table 3. Nei and Shannon index of different Korean pine populations**

Populations	Size	Nei index	Shannon index
Vladivostok	18	0.2118	0.3225
Tangwanghe	26	0.3021	0.4583
Heihe	20	0.2402	0.3617
Erdaobaihe	23	0.2613	0.3951

### Interspecific and intraspecific differentiations of genetic diversities in natural Korean pine populations

The genetic diversities between interspecies and intraspecies at different amplification sites of different primers had significant differences according to Nei index. The value of interspecific total genetic diversities (*H<sub>t</sub>*) ranged between 0.0596 and 0.5000, with an average of 0.3477. The value of interspecific genetic diversities (*H<sub>s</sub>*) ranged between 0.0539 and 0.4909, with an average of 0.2538. Intraspecific relative amount of genetic differentiation (*G<sub>st</sub>*) had a bigger range, from 0.0008 to 0.7327, with an average of 0.2700 (Nei 1972). Interspecific and intraspecific genetic diversities were 73% and 27% of total genetic diversity, respectively (Table 4). It indicated that genetic diversities of Korean pine populations mainly came from the interspecies.

### Genetic distances of different Korean pine populations

Genetic distance (*D*) is the function of dominant gene frequency. The genetic distance between Erdaobaihe and Heihe was smallest (0.0865) and biggest (0.3223) between Vladivostok and Heihe (Table 5). The values of genetic identity ranged between 0.9171 and 0.7145 of four populations. The values of genetic identity of Heihe and Erdaobaihe populations were very close, but those of Heihe and Vladivostok populations had most significant difference. It suggested that there was no correlation between genetic distance and geographical distance for four Ko-

rean pine populations.

**Table 4. Interspecific and intraspecific genetic diversities and genetic differentiations of Korean pine analyzed by Nei index**

Loci	Ht	Hs	Gst	Loci	Ht	Hs	Gst
834-1	0.0596	0.0539	0.0951	812-1	0.3025	0.2464	0.1854
834-2	0.4406	0.1298	0.7055	812-2	0.0596	0.0539	0.0951
853-1	0.2647	0.2587	0.0226	812-3	0.4920	0.3366	0.3159
853-2	0.4843	0.4430	0.0853	812-4	0.4362	0.1223	0.7195
818-1	0.3835	0.3682	0.0398	812-5	0.4620	0.1237	0.7323
818-2	0.1110	0.0986	0.1113	812-6	0.3511	0.2528	0.2799
818-3	0.4982	0.4909	0.0147	811-1	0.4731	0.3274	0.3079
818-4	0.5000	0.4188	0.1624	811-2	0.2436	0.1947	0.2006
818-5	0.1778	0.1707	0.0396	811-3	0.4547	0.2862	0.3706
818-6	0.2289	0.2271	0.0081	811-4	0.3188	0.3185	0.0008
818-7	0.4881	0.2310	0.5267	807-1	0.3047	0.2913	0.0438
808-1	0.4968	0.2188	0.5595	807-2	0.3943	0.3738	0.0520
808-2	0.3116	0.2646	0.1508	827-1	0.4354	0.3072	0.2944
848-1	0.3314	0.3151	0.0492	827-2	0.4650	0.1592	0.6577
848-2	0.4468	0.3550	0.2055	827-3	0.2210	0.1993	0.0983
848-3	0.2661	0.2503	0.0593	827-4	0.0808	0.0770	0.0476
828-1	0.4544	0.2086	0.5409	827-5	0.1323	0.1204	0.0894
828-2	0.4863	0.2339	0.5191	827-6	0.1316	0.1209	0.0815
828-3	0.2788	0.1886	0.3235	827-7	0.4537	0.3622	0.2017
828-4	0.5000	0.4358	0.1283	827-8	0.4905	0.3354	0.3163
823-1	0.1205	0.1141	0.0527	827-9	0.4999	0.1642	0.6715
823-2	0.0884	0.0791	0.1051	827-10	0.4815	0.4467	0.0722
823-3	0.4719	0.2066	0.5623	820-1	0.4524	0.4463	0.0135
836-1	0.1432	0.1343	0.0617	820-2	0.4995	0.4524	0.0943
836-2	0.3328	0.3082	0.0739	820-3	0.4989	0.2202	0.5586
836-3	0.0453	0.0438	0.0336	873-1	0.4963	0.4492	0.0948
836-4	0.2819	0.2686	0.0472	Mean	0.3477	0.2538	0.2700
822-1	0.4536	0.4034	0.1107	St.Dev	0.0224	0.0148	

Ht: Interspecific total genetic diversity, Hs: Interspecific genetic diversity, Gst: Intraspecific relative amount of genetic differentiation

**Table 5. Genetic distances and coherences in the four Korean pine populations**

Populations	Vladivostok	Tangwanghe	Heihe	Erdaobaihe
Vladivostok	****	0.8797	0.7245	0.7866
Tangwanghe	0.1281	****	0.7995	0.8958
Heihe	0.3223	0.2238	****	0.9171
Erdaobaihe	0.2400	0.1100	0.0865	****

## Discussion

The ratio of polymorphic site is 60.7% for 87 amplification products with 15 primers. Each primer averaged 3.6 polymorphic sites. It is hard to compare the genetic diversity of Korean pine with other gymnosperm close to Korean pine because ISSR technique was seldom used in the field. Tsumura *et al* (1996) studied *Pseudotsuga menziesii* and *Cryptomeria japonica* by the ISSR and found each primer averaged 2.32 and 1.77 polymorphic sites, respectively, which were significantly lower than that of Korean pine. Xia *et al.* (2001) reported that the genetic diversity of Korean pine was close to the intermediate level of *Pinaceae* plant by RAPD technique. Zu *et al.* (1989) also proved genetic diversity level of Korean pine was higher than those of other *Pinus* plants by the analysis of allozyme. Thus, our results were consistent with them. It indicated that genetic diversity of Korean pine was not low at least. Korean pine, as age-old tree species, accumulated plentiful variations during the long-term evolution since the tertiary. Korean pine was longevity,

out-crossing and wind pollination plant, whose life cycle was K strategy and the genetic system was flexible (Stebbins 1950). These characteristics are beneficial to improve the number of gene recombination. Thus the level of genetic diversity of Korean pine is higher. However, long sex-mature period and low seed yield limit the recombination of gene to some extent, which affect the level of genetic diversity of Korean pine.

The ratio of polymorphic site of population was highest in Tangwanghe, secondly in Changbai Mountains and Heihe, and lowest in Vladivostok. The genetic diversity showed the same change trend as the polymorphic site according to the Nei and Shannon indexes. Korean pine mainly distribute in Changbai Mountain and Xiaoxing'an Mountains. Heihe lies to the north-west border of the distribution of Korean pine, and Vladivostok is situated on the south border of the range of Korean pine in Far East (Ma 1992). It suggested that the genetic diversity of Korean pine in center zone was higher compared with that in edge zone. The differences in genetic diversity in different populations were resulted from the distribution and evolution. As the survivor in tertiary, Korean pine extended from Korean peninsula, where was ever the refuge, to Xiaoxing'an Mountains via Changbai Mountain after ice age (Ma *et al.* 1997). There were higher population density and chance of amphimixis at random, thus with a higher genetic diversity in the center of distribution. The smaller number and density decreased the probability of gene combination in the fringe region, which resulted in 'necking effect' and 'founder effect', and decline of level of genetic diver-

sity (Peng *et al.* 1997).

Genetic diversities within and among the populations of Korean pine were 73% and 27% of total genetic diversity, respectively. It indicated that the variation mainly came from the interior of the population, which was consistent with mostly wind and cross pollination woody plants. In general, genetic variation among populations accounted for 10% for wind and cross pollination woody plants (Hamrick and Godt 1990). The reasons why our results were higher than this average were longer geographical distance among four populations and natural selection of environment. Different ecological microenvironment could cause the significant difference in genetic structure (Su *et al.* 1997; Taylor and Aarssen 1990). Thus, there was ecological segregation among the four populations of Korean pine.

Korean pine and its relatives (i.e. *Pinus pumila*) present an overlapped distribution in the experimental sites. It is only a scientific hypothesis that intercross of interspecies between Korean pine and its relatives resulted in gene flow. Natural hybrid had been found between *Pinus sibirica* and *Pinus pumila*. It indicated that reproductive segregation of interspecies was not strict for five-needle pine (Goroshkevich 1999). The further study on the hypothesis is still debated. The higher genetic differentiation of Korean pine populations could guide to select excellent seeds.

There was no significant correlation between genetic distance and geographical distance for the four Korean pine populations, which was related to specific region and environment, handicap of gene flow caused by segregation in interspecies grown nearly, different seed source, formation system and evolution.

The gradual decrease in distribution region of Korean pine populations was a result from human destroy and environmental factors (i.e. fire and wind throw), rather than the lower genetic diversity. Thus, the strong emphases for Korean pine protection will be put on the decreasing human disturbance.

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